Pharmacology, Biochemistry and Behavior 98 (2011) 304–310

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Tamoxifen effects on respiratory chain complexes and creatine kinase activities in an animal model of mania

Morgana Moretti [a], Samira S. Valvassori [a], Amanda V. Steckert [a], Natalia Rochi [b], Joana Benedet [b], Giselli Scaini [b], Flávio Kapczinski [c], Emilio L. Streck [b], Alexandra I. Zugno [b], João Quevedo [a,d,*]

[a] Laboratory of Neurosciences and National Institute for Translational Medicine (INCT-TM), Postgraduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, 88806-000 Criciúma, SC, Brazil

[b] Laboratory of Experimental Pathophysiology and National Institute for Translational Medicine (INCT-TM), Postgraduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, 88806-000 Criciúma, SC, Brazil

[c] Bipolar Disorders Program, Laboratory of Molecular Psychiatry and National Institute for Translational Medicine (INCT-TM), Federal University of Rio Grande do Sul, 90035-003 Porto Alegre, RS, Brazil

[d] Laboratory of Translational Psychiatry, Research Center, São José Hospital, 88801-250 Criciúma, SC, Brazil

ARTICLE INFO

Article history:
Received 17 October 2010
Received in revised form 27 December 2010
Accepted 23 January 2011
Available online 28 January 2011

Keywords:
Mania
Locomotor activity
Mitochondrial respiratory chain
Creatine kinase
Tamoxifen

ABSTRACT

The present study aimed to investigate the effects of tamoxifen (TMX) on locomotor behavior and on the activities of mitochondrial respiratory chain complexes and creatine kinase (CK) in the brain of rats subjected to an animal model of mania induced by D-amphetamine (D-AMPH)—reversion and prevention protocols. The D-AMPH administration increased locomotor activity in saline-treated rats under prevention and reversion treatment; furthermore, there was evident reduction in the locomotion in the D-amphetamine group treated with TMX. D-AMPH significantly decreased the activity of mitochondrial respiratory chain complexes in saline-treated rats in prefrontal cortex, hippocampus, striatum and amygdala in both prevention and reversion treatment. Depending on the cerebral area and evaluated complex, TMX was able to prevent and reverse this impairment. A decrease in CK activity was also verified in the brain of rats when D-AMPH was administrated in both experiments; the administration of TMX reversed but not prevented the decrease in CK activity induced by D-AMPH. The present study demonstrated that TMX reversed and prevented the alterations in behavioral and energy metabolism induced by D-AMPH (alterations were also observed in bipolar disorder), reinforcing the need for more studies about inhibitors of PKC as possible targets for new medications in the treatment of bipolar disorder.

1. Introduction

Bipolar disorder (BD) is a common and severe mood disorder that causes recurrent mood switches including manic, depressive, and mixed episodes (Manning et al., 1997). This condition is associated to emotionally damaging behaviour and bears a 15% risk of suicide when left untreated (McIntyre et al., 2008).

The mania, considered the clinical hallmark of the disease, shows symptoms such as euphoria, excessive energy, grandiosity, acceleration, intense sensation of pleasure or a highly irritable and aggressive state (Calabrese et al., 2003). The treatment of acute mania with lithium, valproate, carbamazepine and atypical antipsychotics has shown great progress, however many patients do not tolerate or respond adequately to these drugs (Evins et al., 2006). In addition, these treatments necessitate continuous long-term use and are thus non-curative (Ludtmann et al., 2011), requiring new approaches in BD pharmacotherapy.

Evidences from literature strongly suggest a dysregulation of mitochondrial function and energetic metabolism in the pathophysiology of neuropsychiatric disorders, including BD (Kato and Kato, 2000; Konradi et al., 2004; Quiroz et al., 2008). In this context, in vivo magnetic resonance spectroscopy studies have demonstrated changes in brain compounds related to energy production, oxidative phosphorylation and phospholipid metabolism in bipolar patients (Stork and Renshaw, 2005). Moreover, neuroimaging studies (Cecil et al., 2002; Bertolino et al., 2003; Deicken et al., 2003), postmortem brain (Konradi et al., 2004; Vawter et al., 2006), and animal models (Corrêa et al., 2007; Valvassori et al., 2010) suggest that the decrease in mitochondrial function with consequent impairment of cell energy production is a hypothesis which might explain the pathophysiology of BD. These observations suggest that enhancing mitochondrial

* Corresponding author at: Laboratório de Neurociências, PPGCS, UNASAU, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil. Tel.: +55 48 3431 2578; fax: +55 48 3443 4817.
E-mail address: quevedo@unesc.net (J. Quevedo).

0091-3057/
doi:10.1016/j.pbb.2011.01.017

function may represent an important strategy for the optimal long-term treatment of BD (Quiroz et al., 2008).

Creatine kinase (CK) is an enzyme that plays a central role in cells with high and fluctuating energy requirements, including neurons (Andres et al., 2008), where it acts as an effective buffering system of cellular ATP levels. A study by MacDonald et al. (2006) showed that the expression levels of mRNA transcripts coding for brain creatine kinase and mitochondrial 1 ubiquitous are downregulated in the hippocampus and dorsolateral prefrontal cortex in the postmortem brain of bipolar patients. Additionally, in an animal model of mania, was demonstrated that the levels of CK are decreased in brain tissue (Streck et al., 2008).

Several lines of evidence also implicate abnormal protein kinase C (PKC) activity in BD. This protein is mainly found in the brain, where it exerts fundamental role in pre and post-synaptic neurotransmission, regulating neuronal excitability, neurotransmitter release and cellular plasticity (Manji and Lenox, 1999). Psychostimulant drugs seem to activate PKC (Einat et al., 2007; Boudanova et al., 2008) and evidences suggest that lithium and valproate attenuate the function of this protein, indicating that PKC modulation plays a key role in the treatment of mania (Chen et al., 2000; Kirshenboim et al., 2004; Hahn et al., 2005). Recent preclinical and clinical studies have demonstrated that tamoxifen (TMX—an inhibitor of PKC) significantly reduces hyperactivity and risky behavior caused by D-AMPH administration in animals and also reduces manic symptoms in patients (Bebchuk et al., 2000; Einat et al., 2007; Zarate et al., 2007; Yildiz et al., 2008), suggesting efficacy of TMX in the treatment of mania. In addition, Tuquet et al. (2000) demonstrated that TMX interacts with mitochondrial respiratory chain in the isolated mitochondria from rat liver, suggesting a correlation between PKC inhibition and increased energy metabolism.

The fluctuating pattern of BD hampers the development of a suitable animal model that includes all the symptoms of the disorder. The clinical hallmark for the diagnosis of BD is the presence of manic symptoms (Belmaker, 2004) so the usual animal models for BD are focused on the expression of a single manic episode. The psychostimulant-induced hyperactivity is the best established animal model of mania (Machado-Vieira et al., 2004). It causes hyperlocomotion, insomnia and increased sexual drive, representing face validity (Fiorino and Phillips, 1999), it reproduces pathophysiological characteristics of the human condition (construct validity) (Frey et al., 2006d), and responses to antimanic agents, such as antipsychotics and mood stabilizers (predictive validity) (Frey et al., 2006b,c).

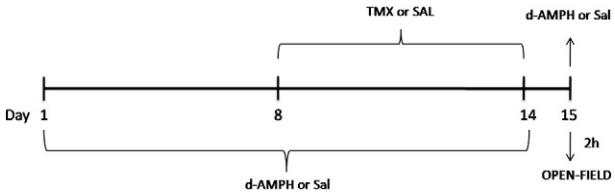
In this context, the present study aimed to investigate the effects of tamoxifen on locomotor behavior, on the activity of mitochondrial respiratory chain complexes and on CK activity in brains of rats subjected to an animal model of mania induced by D-AMPH.

2. Materials and methods

2.1. Animals

Adults male Wistar rats (250–300 g) were obtained from the Central Animal House of Universidade do Extremo Sul Catarinense. These animals were maintained on a 12-h light–dark cycle (lights on at 7:00 am), at a temperature of 23 °C ± 1 °C and with free access to food and water. These conditions were maintained constant throughout the experiments. The rats were caged in groups of five in a 41 × 34 × 16 cm cage and divided randomly in 12 animals per group, totaling 96 animals. All behavioral data were conducted in a calm room by an experienced observer. The observer was in the room where experiments were performed and was blind to the animal condition.

All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of the local Ethics Committee of Animals Use.



Scheme 1. Treatment regime in reversal protocol.

2.2. Drugs

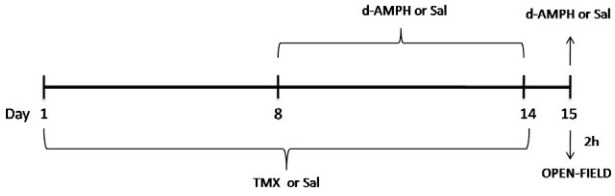
Tamoxifen citrate (Salutas Pharma GmbH, Barleben, Ger.) and D-amphetamine (Sigma, St Louis, Mo.) were directly dissolved in saline (Sal) solution (NaCl 0.9%, w/v) and used in doses based on previous studies (TMX Einat et al., 2007; D-AMPH—Frey et al., 2006c).

2.3. Reversal treatment

In the reversal model, we reproduced the treatment of acute manic episode according previously proposed (Frey et al., 2006c). Rats received intraperitoneal (i.p.) injection of either D-AMPH (2 mg/kg) or Sal (1 mL/kg) once a day for a period of 14 days. From the 8th to the 14th day (treatment for 7 days), D-AMPH and Sal treated animals also received Sal (1 mL/kg i.p.—twice a day) or TMX (1 mg/kg i.p.—twice a day), totaling four experimental groups of 12 animals per group: Sal + Sal, Sal + TMX, D-AMPH + Sal and D-AMPH + TMX. No behavioral assessment was performed between days 1–14. On the 15th day of treatment, the animals received a single injection of D-AMPH or Sal and locomotor activity was assessed using the open-field test 2 h after the injection, since pharmacokinetics studies have shown that half life of D-AMPH in the body is 60 min (Honecker and Coper, 1975). The rats were killed by decapitation immediately after the open-field test and prefrontal cortex, hippocampus, striatum and amygdala were manually dissected on ice, rapidly frozen on dry ice and stored at −70 °C until assayed (See Scheme 1).

2.4. Prevention treatment

This protocol was designed to mimic the prevention phase of BD treatment, as previously proposed (Frey et al., 2006c). Rats received either Sal (1 mL/kg i.p.—twice a day) or TMX (1 mg/kg i.p.—twice a day) for a period of 14 days. From the 8th to the 14th day (treatment for 7 days), TMX and Sal treated animals also received Sal (1 mL/kg i.p.—once a day) or D-AMPH (2 mg/kg—once a day), totaling four experimental groups of 12 animals per group: Sal + Sal, TMX + Sal, Sal + D-AMPH and TMX + D-AMPH. No behavioral assessment was performed between days 1 and 14. On the 15th day of treatment, the animals received a single injection of D-AMPH or Sal and locomotor activity was assessed using the open-field test 2 h after the injection. The rats were killed by decapitation immediately after the open-field test and prefrontal cortex, hippocampus, striatum and amygdala were manually dissected on ice, rapidly frozen on dry ice and stored at −70 °C until assayed (See Scheme 2).



Scheme 2. Treatment regime in prevention protocol.

2.5. Open-field test

On the 15th day of treatment, the animals received a single injection of D-AMPH or Sal, and 2 h after the injection locomotor activity was measured using the open-field test. The test was performed in a 40×60 cm open field surrounded by 50 cm high walls. The floor of the apparatus was constructed with varnished wood and divided into 9 equal rectangles by black lines. The animals were gently placed in one of the corner squares at the start of the test in order to explore the arena for 5 min. Crossings of the black lines and rearings (exploratory activity) were counted.

2.6. Activity of mitochondrial respiratory chain enzymes

Prefrontal cortex, hippocampus, striatum and amygdala were homogenized (1:10, w/v) in SETH buffer (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin, pH 7.4). The homogenates were centrifuged at 800 *g* for 10 min and the supernatants were used for determining the activities of the mitochondrial respiratory chain enzymes (complexes I, II, III and IV). On the day of the assays, the samples were frozen and thawed thrice in hypotonic assay buffer to fully expose the enzymes to substrates and achieve maximal activities. NADH dehydrogenase (complex I) was evaluated by the method described by Cassina and Radi (1996) by the rate of NADH-dependent ferricyanide reduction at l = 420 nm. The activities of succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) and succinate: Cytochrome c oxidoreductase (complexes II–III) were determined by the method described by Fischer et al. (1985). Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at l = 600 nm. Complex II–III activity was measured by cytochrome c reduction from succinate at l = 550 nm. The activity of cytochrome c oxidase (complex IV) was assayed according to the method described by Rustin et al. (1994), measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome c at l = 550 nm. The activities of themitochondrial respiratory chain complexes were calculated as nmol/min mg protein.

2.7. Creatine kinase activity

Creatine kinase (CK) activity was assessed in brain homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM of phosphocreatine, 9 mM of $MgSO_4$ and approximately 0.4–1.2 μg of protein in a final volume of 100 μL. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 3.2 mmol of ADP plus 0.8 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 μmol of *p*-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). The coloring was developed through the addition of 100 μL of 2%α-naphtol and 100 μL of 0.05% diacetyl in a final volume of 1 mL and read spectrophotometrically after 20 min at 540 nm. Results were expressed as mmol of creatine formed per min per mg protein.

2.8. Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey test when p values were significant ($p<0.05$). All analyses were performed using the Statistical Package for the Social Science (SPSS) version 17.0 software.

3. Results

3.1. Reversal model

As illustrated in Fig. 1, D-AMPH administration increased crossings and rearings in saline-treated rats and this effect was reversed by TMX administration. TMX alone did not alter behavioral parameters in this protocol of treatment.

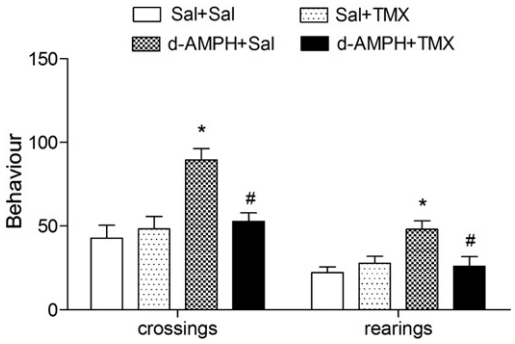


Fig. 1. Numbers of crossings and rearings in reversal model. (n = 12 for each group). Data were analyzed by two-way analysis of variances followed by Tukey test when *p* was significant. Values are expressed as mean ± S.E.M. * $p<0.05$ difference of Sal + Sal group. # $p<0.05$ difference of D-AMPH + Sal group. Bars represent means; error bars represent standard error of means.

As shown in Fig. 2, in the reversal treatment TMX administration in Sal-pretreated groups did not modify the complex viability (Fig. 2A, C and D), except in hippocampus, where TMX decreased complex II activity (Fig. 2B). The administration of D-AMPH resulted in a marked inhibition of complexes I (Fig. 2A), II (Fig. 2B), III (Fig. 2C) and IV (Fig. 2D) of mitochondrial respiratory chain in prefrontal cortex, hippocampus, striatum and amygdala. In the hippocampus and striatum the TMX administration partially reversed the decrease of complex II activity induced by D-AMPH (Fig. 2B). On the other hand, the inhibition of complex IV was totally reversed by this drug in all brain regions analyzed (Fig. 2D). Conversely, TMX did not modified the decreased activity of complex I (Fig. 2A) and III (Fig. 2C) in the D-AMPH-treated group.

D-AMPH administration significantly inhibited CK activity in the rat's hippocampus and striatum, but not in the amygdala and prefrontal cortex (Fig. 3). The administration of TMX reversed D-AMPH-induced inhibition of CK activity in hippocampus and striatum. Besides, in this protocol of treatment CK activity was significantly increased by the treatment with TMX in D-AMPH-groups in the prefrontal cortex of the rats.

3.2. Prevention model

Fig. 4 summarizes the results of locomotor activity in the prevention model. Crossings and rearings were significantly increased by D-AMPH administration in saline-treated rats and TMX prevented D-AMPH-related hyperactivity.

Results for mitochondrial respiratory chain complexes I, II, III and IV activity are shown in Fig. 5. The treatment with Sal plus TMX decreased the activity of complex II in amygdala and complex IV in striatum. The administration of D-AMPH in the prevention protocol also resulted in a marked inhibition of complexes I (Fig. 5A), II (Fig. 5B), III (Fig. 5C) and IV (Fig. 5D) of mitochondrial respiratory chain in prefrontal cortex, hippocampus, striatum and amygdala. The inhibition of complexes I and III was totally prevented by TMX in all brain regions analyzed (Fig. 5A and C, respectively). Furthermore, TMX totally prevented D-AMPH-induced inhibition of complex II in prefrontal cortex and amygdala, but no effect of TMX on the mitochondrial alteration in the hippocampus and striatum was observed (Fig. 5B). The administration of TMX significantly decreased the D-AMPH-induced complex IV inhibition in the prefrontal cortex, hippocampus and amygdale. In addition, TMX administration decreased partially the inhibition of complex IV activity in striatum (Fig. 5D).

In the treatment of prevention, TMX alone decreased CK activity in amygdala, hippocampus and striatum, but not in the prefrontal cortex

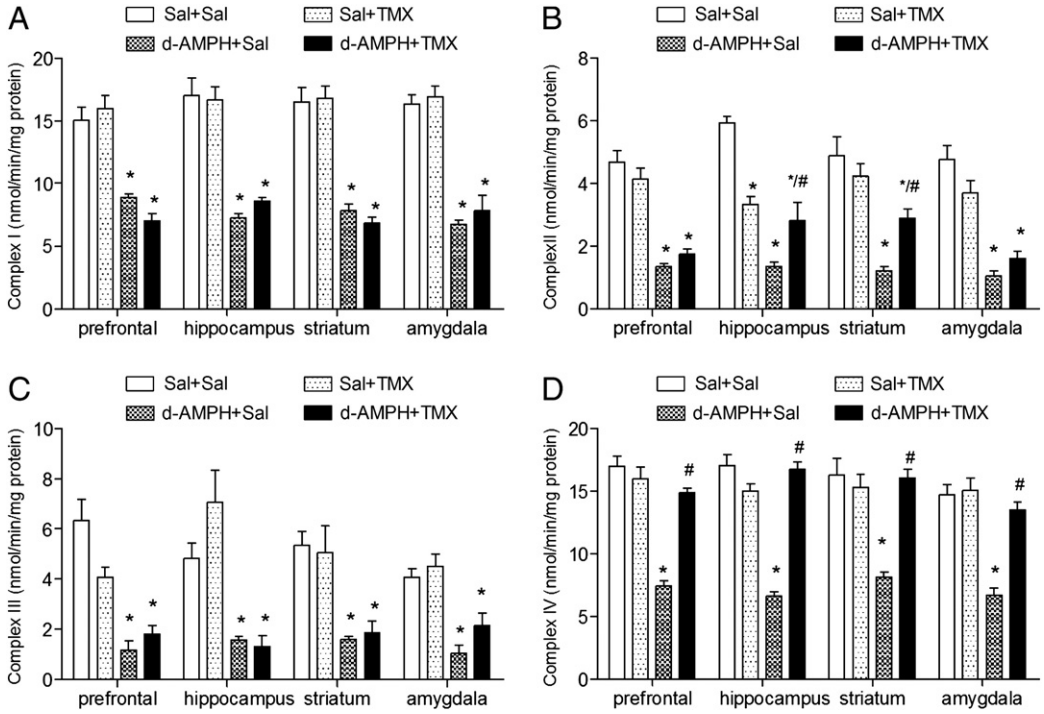


Fig. 2. Numbers of crossings and rearings in prevention model. (n = 12 for each group). Data were analyzed by two-way analysis of variances followed by Tukey test when *p* was significant. Values are expressed as mean ± S.E.M. * $p<0.05$ difference of Sal + Sal group. # $p<0.05$ difference of D-AMPH + Sal group. Bars represent means; error bars represent standard error of means.

when compared to the control groups (Fig. 6). Moreover, the administration of D-AMPH resulted in a marked inhibition of CK activity in the amygdala, hippocampus and striatum. The same was not observed in the prefrontal cortex. In the hippocampus and amygdala, the pretreatment with TMX potentiates the inhibition of CK activity induced by AMPH. In addition, CK activity inhibition induced by D-AMPH was not altered by the pretreatment with TMX in the striatum of the rats. In the prefrontal cortex, the pretreatment with TMX decreased CK activity in the D-AMPH-group.

4. Discussion

D-AMPH has profound effects on a wide range of physiological and behavioral processes: motor activity, ingestive behavior, sleep, attention, aggression, sexual behavior, learning and memory, classical conditioning and operant behavior (Seiden et al., 1993). Psychostimulant-induced hyperactivity is the best established animal model of mania (Machado-Vieira et al., 2004). The hyperactivity induced by D-AMPH has been reported by several authors (Cappeliez and Moore, 1990; Einat et al., 2007; Szabo et al., 2009; Young et al., 2010) and this effect is mediated by an increased dopaminergic transmission in some brain regions, moreover, antipsychotic drugs used to treat acute mania are dopaminergic receptor antagonists (Meyer and Quenzer, 2005; Kapczinski and Quevedo, 2009). So, the data of the present study are in line with previous findings which showed consistent hyperlocomotor effects of D-AMPH in rats (Frey et al., 2006a,b,c,d; Mavrikaki et al., 2010; Valvassori et al., 2010).

The reversion and prevention of D-AMPH-induced hyperlocomotion were seen after treatment with TMX, whose main action is to inhibit PKC, besides, the groups given TMX plus Sal did not differ from the control groups (Sal + Sal), indicating that the effects of TMX could not be attributed to sedation. This finding confirms previous studies showing that TMX reduced the hyperactivity induced by D-AMPH in the open-field test (Einat et al., 2007). Literature data also show that another

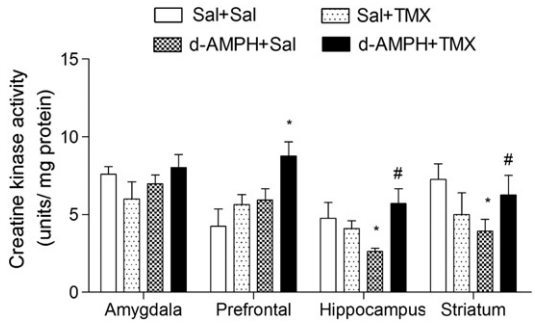


Fig. 3. Mitochondrial respiratory chain complexes I, II, III and IV activity in prefrontal cortex, hippocampus, striatum and amygdala in reversal model. (n = 6 for each group). Data were analyzed by two-way analysis of variances followed by Tukey test when *p* was significant. Values are expressed as mean ± S.E.M. * $p<0.05$ difference of Sal + Sal group. # $p<0.05$ difference of D-AMPH + Sal group. Bars represent means; error bars represent standard error of means.

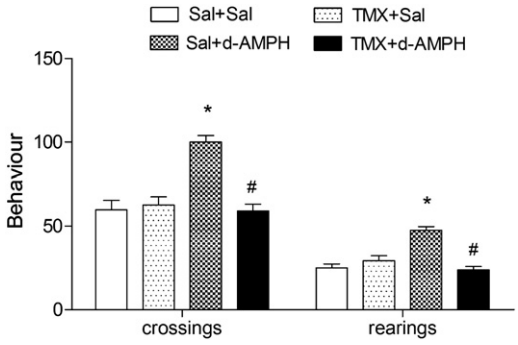


Fig. 4. Mitochondrial respiratory chain complexes I, II, III and IV activity in prefrontal cortex, hippocampus, striatum and amygdala in prevention model. (n = 6 for each group). Data were analyzed by two-way analysis of variances followed by Tukey test when *p* was significant. Values are expressed as mean ± S.E.M. * $p<0.05$ difference of Sal + Sal group. # $p<0.05$ difference of D-AMPH + Sal group. Bars represent means; error bars represent standard error of means.

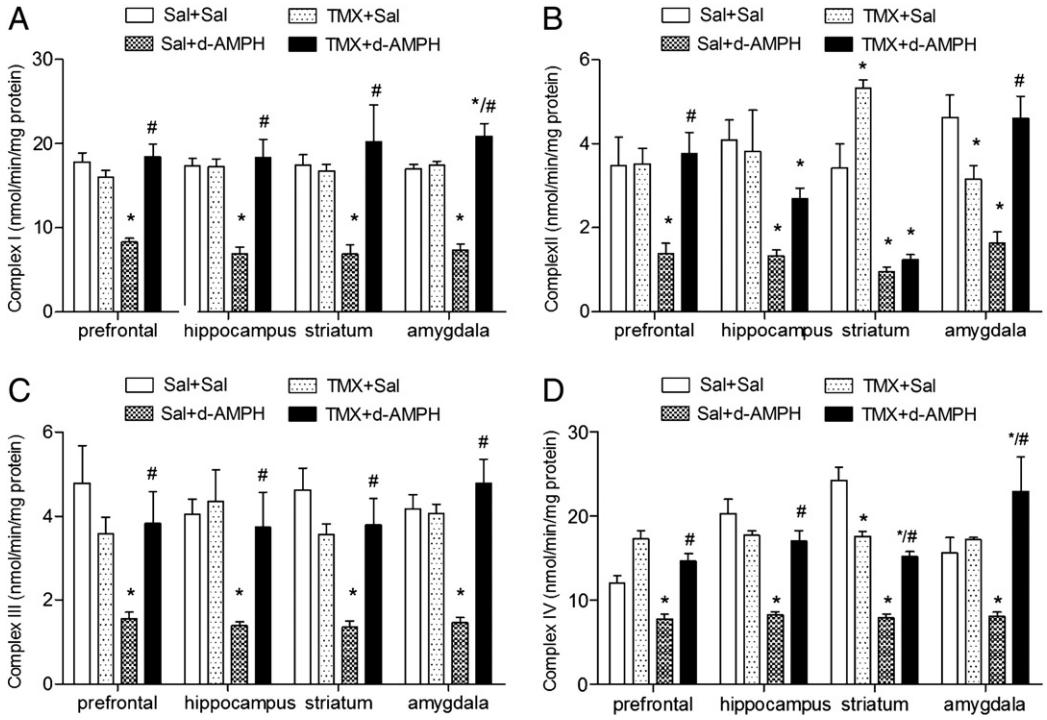


Fig. 5. Creatine kinase (CK) activity in the amygdala, prefrontal cortex, hippocampus and striatum of rats following reversal treatment (n = 5 for each group). Data were analyzed by two-way analysis of variances followed by Tukey test when p was significant. Values are expressed as mean ± S.E.M. * $p<0.05$ difference of Sal + Sal group. # $p<0.05$ difference of D-AMPH + Sal group. Bars represent means; error bars represent standard error of means.

inhibitor of PKC, Ro31-8220, when administered in the nucleus accumbens, attenuates the motor response produced by D-AMPH, an effect which was associated with the blocking of the dopamine release (Browman et al., 1998). Additionally, Einat et al. (2007) showed that the administration of D-AMPH increased phosphorylation of GAP-43 protein—implicated in neuronal differentiation, plasticity and neurotransmitter release—and that pretreatment with TMX eliminates the effect of D-AMPH in this protein phosphorylation in parallel with behavioral changes, reinforcing the importance of modulation of PKC in the treatment of mania.

However, it is important to consider that TMX is also an estrogenic receptor modulator (with estrogenic and antiestrogenic effects), which could contribute to its behavioural effect. In this context, a study evaluated whether the antimanic effect of TMX is mediated through the PKC inhibitory and/or the antiestrogenic action(s) of the drug. TMX and chelerythrine (a PKC inhibitor) completely blocked the hyperlocomotion induced by D-AMPH. However, while the intermediate medroxyprogesterone (an antiestrogenic drug) dose partially reduced the D-AMPH-induced hyperlocomotion, lower and high doses produced no effect (Sabioni et al., 2008), indicating a major role for PKC inhibition in the antimanic-like effect of TMX.

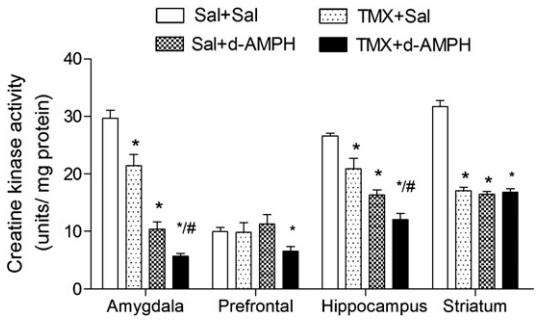


Fig. 6. Creatine kinase (CK) activity in the amygdala, prefrontal cortex, hippocampus and striatum of rats following prevention treatment (n = 5 for each group). Data were analyzed by two-way analysis of variances followed by Tukey test when p was significant. Values are expressed as mean ± S.E.M. * $p<0.05$ difference of Sal + Sal group. # $p<0.05$ difference of D-AMPH + Sal group. Bars represent means; error bars represent standard error of means.

Our results also showed that D-AMPH administration resulted in an inhibition of complexes I, II, III and IV of mitochondrial respiratory chain in prefrontal cortex, hippocampus, striatum and amygdala, confirming previous studies that demonstrated a dysfunction in mitochondrial function during mania (Andreazza et al., 2010).

Several evidences have shown that the activation of PKC enhances the release of dopamine, a neurotransmitter implicated in the manic syndrome (Robinson, 1991; Cowell et al., 2000;) and that PKC inhibition reduces dopamine release induced by D-AMPH (Giambalvo, 1992; Kantor and Gnegy, 1998). In this context, Przedborski et al. (1993) showed that the administration of levodopa (L-DOPA), the precursor of dopamine, was able to inhibit the activity of complex I in rat brains. This group also demonstrated that the incubation of mitochondria with dopamine and L-DOPA inhibited the activity of complex I in a time-dependent way, strengthening the link between dopaminergic hyperactivity and mitochondrial damage. Moreover, high levels of DA are related to increased cerebral oxidative stress and damage to mitochondrial function due to metabolism of oxidation of this monoamine (Kapczinski and Quevedo, 2009). In addition, it is important to emphasize that the increased activity of PKC induced by the administration of D-AMPH is related to an increased Ca^{2+} influx (Yang and Kazanietz, 2003). A large movement of positive charges of Ca^{2+} into mitochondria increases the permeability of the mitochondrial membrane, decreasing its potential and exerting a depolarising effect (Quiroz et al., 2008). These aspects are linked with the interruption of oxidative phosphorylation and production of ATP, with consequent release of cytochrome c and cell death by apoptosis (Rasola et al., 2010). Together with our results, these studies suggest that inhibition of PKC, with consequent decrease in dopamine release is able to protect mitochondrial respiratory chain complexes viability against damage induced by D-AMPH.

Here, we found that the inhibition of the mitochondrial respiratory chain complexes was prevented by TMX, however it varies depending

Table 1
Summary of tamoxifen effects on D-AMPH-induced behavioral and biochemical alteration in an animal model of mania-reversal treatment. ↓ = decrease; Rev = Reversion of D-AMPH effects; PC = Prefrontal cortex; HIP = Hippocampo; STR = Striatum; AMY = Amygdala.

	Locomotor activity	Complex I activity	Complex II activity	Complex III activity	Complex IV activity	CK activity	Brain structures
D-AMPH	↑	↓	↓	↓	↓	–	PC
		↓	↓	↓	↓	↓	HIP
		↓	↓	↓	↓	↓	STR
		↓	↓	↓	↓	–	AMY
TMX	Rev	–	–	–	–	–	PC
		–	Rev	–	–	Rev	HIP
		–	Rev	–	–	Rev	STR
		–	–	–	–	–	AMY

on the protocol of treatment, brain region and complex activity evaluated, which may have occurred due to the longer treatment with SB in the reversal model when compared to the prevention protocol. Furthermore, regions of the central nervous system can respond distinctly (Sullivan et al., 2005), and the activity of mitochondrial respiratory chain complexes was analyzed in different brain regions, which in part represent different cell types, indicating heterogeneity in terms of physiological and metabolic characteristics (Lai et al., 1977; Sims, 1991; Sonnewald et al., 1998). Another interesting fact is that the treatment with Lithium and valproate—both PKC inhibitors—reverses the inhibition of mitochondrial respiratory chain complexes induced by D-AMPH (Valvassori et al., 2000), results that are in agreement with those obtained in this study.

We also demonstrated that D-AMPH administration significantly inhibited CK activity in rat hippocampus and striatum. These results are consistent with a previous study of our research group (Streck et al., 2008). In a postmortem study MacDonald et al. (2006) also demonstrated that levels of CK mRNA are decreased in BD patients, especially in the hippocampus. Together these studies suggest that the decrease in CK may have a role in BD.

Here we showed that TMX reverses the D-AMPH-induced CK activity inhibition. Conversely, the administration of lithium and valproate—mood stabilizers that inhibit PKC—in D-AMPH-treated animals did not reverse the inhibition of the CK activity (Streck et al., 2008). This discrepancy may be due to the fact that lithium and valproate have other mechanisms of action in addition to PKC inhibition, suggesting that specific inhibition of PKC by TMX may be a relation with CK modulation. Additionally, it is important to emphasize that TMX acts on estrogen receptor. Recently, studies have found that estrogen receptor agonists and antagonists stimulate energy metabolism, including CK activity (Somjen et al., 2008, 2010, 2011). Together with our findings, these studies suggest that the modulation of CK by TMX can be via estrogen action.

On the other hand, in the prevention model, TMX decreased CK activity, both alone and co-administered with D-AMPH. According to our results, in a study that examined the effects of TMX in rats subjected to myocardial ischemia-reperfusion injury it was observed that the blood levels of CK were reduced after treatment with this drug (Ek et al., 2008). Furthermore, CK activity in homogenate of hearts was inactivated by TMX about 20% at 10 mM of this drug (Miura et al., 2002). This discrepancy may be explained due to the longer treatment with TMX in the prevention model compared to the reversal model, which may be causing adaptations and modulations in CK activity depending on the time of treatment with this drug.

The main limitation of this study is that TMX mechanism of action also involves estrogen receptor modulation, as previously mentioned. However, studies have indicated that this effects may contribute to its antimanic-like action (Sabioni et al., 2008), moreover, it was demonstrated that addition of medroxyprogesterone in drug treatment improves mood in female patients with refractory mood disorder (Chouinard et al., 1987), encouraging the use of antiestrogenic drugs as an another approach in the search for new mood stabilizing drugs.

5. Conclusion

In conclusion, the present study suggests that the mechanism of TMX action involves changes in the mitochondrial respiratory chain complexes and creatine kinase activities in parallel with behavioral changes, thus reinforcing the need for the study of inhibitors of PKC as a possible target for new medications to BD (Tables 1 and 2).

Acknowledgements

This research was supported by grants from CNPq (FK, ELS, AIZ and JQ), FAPESC (JQ), Instituto Cérebro e Mente (JQ) and UNESC (ELS, AIZ and JQ). FK, ELS, AIZ and JQ are CNPq Research Fellows. MM, SSV and GS are holders of a CAPES studentship and CLF and AVS are holders of a CNPq Studentship.

References

Andreazza AC, Shao L, Wang JF, Young LT. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. Arch Gen Psychiatry 2010;67:360–8.
Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in the central nervous system. Brain Res Bull 2008;76:329–43.
Bebchuk JM, Arfken CL, Dolan-Manji S, Murphy J, Hasanat K, Manji HK. A preliminary investigation of a protein kinase C inhibitor in the treatment of acute mania. Arch Gen Psychiatry 2000;57:95–7.
Belmaker RH. Bipolar disorders. N Engl J Med 2004;351:476–86.
Bertolino A, Frye M, Callicott JH, Mattay VS, Rakow R, Shelton- Repella J, et al. Neuronal pathology in the hippocampal area of patients with bipolar disorder: a study with proton magnetic resonance spectroscopic imaging. Biol Psychiatry 2003;53:906–13.

Table 2
Summary of tamoxifen effects on D-AMPH-induced behavioral and biochemical alteration in an animal model of mania-prevention treatment. ↓ = decrease; Prev = Prevention of D-AMPH effects. PC = Prefrontal cortex; HIP = Hippocampo; STR = Striatum; AMY = Amygdala.

	Locomotor activity	Complex I activity	Complex II activity	Complex III activity	Complex IV activity	CK activity	Brain structures
D-AMPH	↑	↓	↓	↓	↓	–	PC
		↓	↓	↓	↓	↓	HIP
		↓	↓	↓	↓	↓	STR
		↓	↓	↓	↓	↓	AMY
TMX	Prev	Prev	Prev	Prev	Prev	–	PC
		Prev	–	Prev	Prev	–	HIP
		Prev	–	Prev	Prev	–	STR
		Prev	Prev	Prev	Prev	–	AMY

Boudanova E, Navaroli DM, Melikian HE. Amphetamine-induced decreases in dopamine transporter surface expression are protein kinase C-independent. Neuropharmacology 2008;54:605–12.
Browman KE, Kantor L, Richardson S, Badiani A, Robinson TE, Gnegy ME. Injection of the protein kinase C inhibitor Ro31-8220 into the nucleus accumbens attenuates the acute response to amphetamine: tissue and behavioral studies. Brain Res 1998;814:112–9.
Calabrese JR, Hirschfiel RM, Reed M. Impact of bipolar disorder on U.S. community sample. J Clin Psychiatry 2003;64:425–32.
Cappeliez P, Moore E. Effect of lithium on an amphetamine animal model of bipolar disorder. Prog Neuropsychopharmacol Biol Psychiatry 1990;14:347–58.
Cassina A, Radi R. Differential inhibitory action of nitric oxide and peroxynitrite onmitochondrial electron transport. Arch Biochem Biophys 1996;328:309–16.
Cecil KM, DelBello MP, Morey R, Strakowski SM. Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. Bipolar Disord 2002;4: 357–65.
Chen G, Masana MI, Manji HK. Lithium regulates PKC-mediated intracellular cross-talk and gene expression in the CNS in vivo. Bipolar Disord 2000;2:217–36.
Chouinard G, Steinberg S, Steiner W. Estrogen-progesterone combination: another mood stabilizer? Am J Psychiatry 1987;144:826.
Corrêa C, Amboni G, Assis LC, Frey BN, Kapczinksi F, Streck EL, et al. Effects of lithium and valproate on hippocampus citrate synthase activity in an animal model of mania. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:887–91.
Cowell RM, Kantor L, Hewlett GH, Frey KA, Gnegy ME. Dopamine transporter antagonists block phorbol ester-induced dopamine release and dopamine transporter phosphorylation in striatal synaptosomes. Eur J Pharmacol 2000;389:59–65.
Deicken RF, Pegues MP, Anzalone S, Feiwell R, Soher B. Lower concentration of hippocampal N-acetylaspartate in familial bipolar I disorder. Am J Psychiatry 2003;160:873–82.
Einat H, Yuan P, Szabo ST, Dogra S, Manji HK. Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. Neuropsychobiology 2007;55:123–31.
Ek RO, Yildiz Y, Cecen S, Yenisey C, Kavak T. Effects of tamoxifen on myocardial ischemia-reperfusion injury model in ovariectomized rats. Mol Cell Biochem 2008;08:227–35.
Evins AE, Demopulos C, Nierenberg A, Culhane MA, Eisner L, Sachs G. A double-blind, placebo-controlled trial of adjunctive donepezil in treatment-resistant mania. Bipolar Disord 2006;8:75–80.
Fiorino DF, Phillips AG. Facilitation of sexual behavior and enhanced dopamine efflux in the nucleus accumbens of male rats after D-amphetamine-induced behavioral sensitization. J Neurosci 1999;1:456–63.
Fischer JC, Ruitenbeek W, Berden JA, Trijbels JM, Veerkamp JH, Stadhouders AM, et al. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 1985;153:23–6.
Frey BN, Andreazza AC, Ceresér KM, Martins MR, Valvassori SS, Réus GZ, et al. Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania. Life Sci 2006a;79:81-286.
Frey BN, Martins MR, Petronilho FC, Dal-Pizzol F, Quevedo J, Kapczinski F. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. Bipolar Disord 2006b;8:275–80.
Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K, et al. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J Psychiatry Neurosci 2006c;31:326–32.
Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K, et al. Changes in antioxidant defense enzymes after D-amphetamine exposure: implications as an animal model of mania. Neurochem Res 2006d;31:699–703.
Giambalvo CT. Protein kinase C and dopamine transport—2. Effects of amphetamine in vitro. Neuropharmacology 1992;31:1211–22.
Hahn CG, Umapathy, Wang HY, Koneru R, Levinson DF, Friedman E. Lithium and valproic acid treatments reduce PKC activation and receptor-G protein coupling in platelets of bipolar manic patients. J Psychiatr Res 2005;39:355–63.
Honecker H, Coper H. Kinetics and metabolism of amphetamine in the brain of rats of different ages. Naunyn-Schmiedebergs Arch Pharmacol 1975;291:111–21.
Hughes BP. A method for estimation for serum creatine kinase reaction and its use in comparing creatine kinase and aldolase activity in normal and pathologic sera. Clin Chim Acta 1962;7:597–604.
Kantor L, Gnegy ME. Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices. J Pharmacol Exp Ther 1998;284:592–8.
Kapczinski F, Quevedo J. Transtorno bipolar: teoria e clínica; 2009. Porto Alegre: Artmed.
Kato T, Kato N. Mitochondrial dysfunction in bipolar disorder. Bipolar Disord 2000;2: 180–90.
Kirshenboim N, Plotkin B, Shlomo SB, Kaidanovich-Beilin O, Eldar-Finkelman H. Lithium-mediated phosphorylation of glycogen synthase kinase-3beta involves PI3 kinase-dependent activation of protein kinase C-alpha. J Mol Neurosci 2004;24: 237–45.
Konradi C, Eaton M, Macdonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. Arch Gen Psychiatry 2004;61:300–8.
Lai YL, Rodarte JR, Hyatt RE. Effect of body position on lung emptying in recumbent anesthetized dogs. J Appl Physiol 1977;43:983–7.
Ludtmann MH, Boeckeler K, Williams RS. Molecular pharmacology in a simple model system: implicating MAP kinase and phosphoinositide signalling in bipolar disorder. Semin Cell Dev Biol 2011;22:105–13.
MacDonald ML, Naydenov A, Chu M, Matzilevich D, Konradi C. Decrease in creatine kinase messenger RNA expression in the hippocampus and dorsolateral prefrontal cortex in bipolar disorder. Bipolar Disord 2006;8:255–64.
Machado-Vieira R, Kapczinski F, Soares JC. Perspectives for the development of animal models of bipolar disorder. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:209–24.
Manji HK, Lenox RH. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of manic-depressive illness. Biol Psychiatry 1999;46: 1328–51.
Manning JS, Haykal RF, Connor PD. On the nature of depressive and anxious states in a family practice setting: the high prevalence of bipolar II and related disorders in a cohort followed longitudinally. Compr Psychiatry 1997;38:102–8.
Mavrikaki M, Nomikos GG, Panagis G. Efficacy of the atypical antipsychotic aripiprazole in D-amphetamine-based preclinical models of mania. Int J Neuropsychopharmacol 2010;5:1–8.
McIntyre RS, Muzina DJ, Kemp DE, Blank D, Woldeyohannes HO, Lofchy J, et al. Bipolar disorder and suicide: research synthesis and clinical translation. Curr Psychiatry Rep 2008;10:66–72.
Meyer JS, Quenzer FL. Psychomotor stimulants: cocaine and the amphetamines. Psychopharmacology: Drugs, The brain, and BehaviorUSA: Sinauer; 2005.
Miura T, Muraoka S, Fujimoto Y. Inactivation of creatine kinase induced by stilbene derivatives. Pharmacol Toxicol 2002;90:66–72.
Przedborski S, Jackson-Lewis V, Muthane U, Jiang H, Ferreria M, Naini AB, et al. Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. Ann Neurol 1993;34:715–23.
Quiroz JA, Gray NA, Kato T, Manji HK. Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacology 2008;33:2551–65.
Rasola A, Sciacovelli M, Pantic B, Bernardi P. Signal transduction to the permeability transition pore. FEBS Lett 2010;584:1989–96.
Robinson PJ. The role of protein kinase C and its neuronal substrates dephosphin, B-50, and MARCKS in neurotransmitter release. Mol Neurobiol 1991;5:87-130.
Rustin P, Chretien D, Bourgeron T, Gerard B, Rötig A, Saudubray JM, et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;228: 35–51.
Sabioni P, Baretta IP, Ninomiya EM, Gustafson L, Rodrigues AL, Andreatini R. The antimanic-like effect of tamoxifen: behavioural comparison with other PKC-inhibiting and antiestrogenic drugs. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1927–31 Epub 2008 Oct 7.
Seiden LS, Sabol KE, Ricaurte GA. Amphetamine: effects on catecholamine systems and behavior. Annu Rev Pharmacol Toxicol 1993;33:639–77.
Sims DE. Recent advances in pericyte biology implications for health and disease. Can J Cardiol 1991;7:431–43.
Somjen D, Tordjman K, Katzburg S, Knoll E, Sharon O, Limor R, et al. Lipoxygenase metabolites are mediators of PTH dependent human osteoblast growth. Bone 2008;42:491–7.
Somjen D, Katzburg S, Knoll E, Sharon O, Posner GH, Stern N. Vitamin D analogs induce lipoxygenase mRNA expression and activity as well as reactive oxygen species (ROS) production in human bone cells. J Steroid Biochem Mol Biol 2010;121:265–7.
Somjen D, Katzburg S, Sharon O, Grafi-Cohen M, Knoll E, Stern N. The effects of estrogen receptors α and β specific agonists and antagonists on cell proliferation and energy metabolism in human bone cell line. J Cell Biochem 2011;112:625–32.
Sonnewald U, Hertz L, Schousboe A. Mitochondrial heterogeneity in the brain at the cellular level. J Cereb Blood Flow Metab 1998;18:231–7.
Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. Mol Psychiatry 2005;10:900–19.
Streck EL, Amboni G, Scaini G, Di-Pietro PB, Rezin GT, Valvassori SS, et al. Brain creatine kinase activity in an animal model of mania. Life Sci 2008;82:424–9.
Sullivan PG, Rabchevsky AG, Waldmeier PC, Springer JE. Mitochondrial permeability transition in CNS trauma: cause or effect of neuronal cell death? J Neurosci Res 2005;79:231–9.
Szabo ST, Machado-Vieira R, Yuan P, Wang Y, Wei Y, Falke C, et al. Glutamate receptors as targets of protein kinase C in the pathophysiology and treatment of animal models of mania. Neuropharmacology 2009;56:47–55.
Tuquet C, Dupont J, Mesneau A, Roussaux J. Effects of tamoxifen on the electron transport chain of isolated rat liver mitochondria. Cell Biol Toxicol 2000;16:207–19.
Valvassori SS, Rezin GT, Ferreira CL, Moretti M, Gonçalves CL, Cardoso MR, et al. Effects of mood stabilizers on mitochondrial respiratory chain activity in brain of rats treated with D-amphetamine. J Psychiatr Res 2010;44:903–9.
Vawter MP, Tomita H, Meng F, Bolstad B, Li J, Evans S, et al. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. Mol Psychiatry 2006;11:663–79.
Yang C, Kazanietz MG. Divergence and complexities in DAG signaling: looking beyond PKC. Trends Pharmacol Sci 2003;24:602–8.
Yildiz A, Guleryuz S, Ankerst DP, Ongür D, Renshaw PF. Protein kinase C inhibition in the treatment of mania: a double-blind, placebo-controlled trial of tamoxifen. Arch Gen Psychiatry 2008;65:255–63.
Young JW, Goey AK, Minassian A, Perry W, Paulus MP, Geyer MA. GBR 12909 administration as a mouse model of bipolar disorder mania: mimicking quantitative assessment of manic behavior. Psychopharmacology (Berl) 2010;208:443–54.
Zarate Jr CA, Singh JB, Carlson PJ, Quiroz J, Jolkovsky L, Luckenbaugh DA, et al. Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study. Bipolar Disord 2007;9:561–70.